REMARKS

I. Rejection of claims 1 and 9 under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1 and 9 under 35 U.S.C. § 103(a) as allegedly being obvious over Whitmarsh et al. (Molecular Human Reproduction, Vol. 2, No. 12, pgs. 911-919) in view of Chapman and Barratt (Molecular Human Reproduction, Vol. 2, No. 10, pgs. 767-777, 1996) and further in view of Franken et al. (Fertility and Sterility, Vol. 66, No. 6, December 1996). The Whitmarsh reference is relied on in the Action for describing a method of measuring the biological activity of recombinant human ZP3 (rhZP3). Chapman and Barratt are relied on in the Action for describing the importance of glycosylation of rhZP3 in sperm binding. Franken is relied on in the Action for describing the expression of human ZP from oocytes derived from postmortem ovarian material. The Examiner asserts that it would have been obvious to utilize a glycosylated rhZP3 as taught by Chapman and Barratt in the method of Whitmarsh to increase sperm binding. The Examiner claims that Whitman in view of Chapman and Barratt differ from the instant invention in not specifically expressing rhZP3 from human ovarian cells, and asserts that Franken's description of the expression of human ZP from oocytes derived from postmortem ovarian material cures this deficiency. Applicants respectfully traverse this rejection because claims 1 and 9 are not obvious over Whitmarsh in view of Chapman and Barratt and further in view of Franken for the reasons discussed below.

To properly make a rejection under 35 U.S.C. § 103, the Examiner has the initial burden of establishing a *prima facie* case of obviousness. Meeting this burden requires the Examiner to show first, that the prior art would have suggested to those of ordinary skill in the art that they

should make the claimed composition or device, or carry out the claimed process. Second, the Examiner must establish that the prior art would have revealed that in so making or carrying out the claimed process, those of ordinary skill in the art would have had a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be found in the prior art, not in Applicants' disclosure. *In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991), citing *In re Dow Chemical Co.*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

Whitmarsh discloses a method of measuring the biological activity of rhZP3. The primary purpose of the Whitmarsh study, as described on pages 911-912, is to use the in vitro transcription and translation system to produce immobilized rhZP3 on agarose beads and to examine the biological activity of the rhZP3 using sperm binding and acrosome reaction data. However, Whitmarsh does not disclose or suggest the use of *properly glycosylated* hZP3 in order to determine human sperm activity. The Examiner asserts that Whitmarsh provides the means by which the rhZP3 can be glycosylated by mentioning the possible incorporation of canine pancreatic microsomal membranes. Applicants respectfully submit that the incorporation of these membranes would not, in fact, result in properly glycosylated, biologically active rhZP3. As described in the present specification, glycosylation is tissue- and species- specific. Furthermore, Applicants have found that only in human cell lines can a properly glycosylated ZP3 protein be produced that has both the sperm-binding activity and acrosome reaction-inducing functions of native hZP3. There is no suggestion in Whitmarsh that the use of canine pancreatic microsomal membranes, as suggested by Whitmarsh, would have resulted in rhZP3 with a correct human glycosylation pattern. In addition, rhZP3 glycopolypeptide produced by

such canine membranes would not possess full biological activity with human sperm in contrast to the rhZP3 of the present invention.

Furthermore, Whitmarsh does not address the importance of properly glycosylated rhZP3. For instance, in observing that the median level of sperm binding to its rhZP3 beads is low, Whitmarsh proposes several reasons on page 916 as to why this may be the case. However, Whitmarsh never suggests that its rhZP3's lack of glycosylation may have been the reason for such low binding results. Similarly, on page 917, Whitmarsh also speculates as to why the acrosome reactions were induced only after long incubation times. Again, Whitmarsh never discloses that the rhZP3's lack of glycosylation could be the reason that such long incubation times were necessary. In fact, Whitmarsh teaches away from the importance of the glycosylation of the human ZP3 protein by contrasting it with the mouse ZP3 protein. As pointed out by the Examiner, Whitmarsh mentions the importance of carbohydrates in the binding of spermatozoa to the mouse zona pellucida at page 917. However, since the rhZP3 in Whitmarsh exhibited some biological activity, while lacking glycosylation, Whitmarsh contrasts human ZP3 with mouse ZP3 in stating:

> The situation in the human is however more complex where the protein backbone also plays a significant role. . . We therefore think that the protein backbone of ZP3 may have a more significant role to play in sperm binding and subsequent acrosome reaction in human than in mouse. We are examining further the role of the protein backbone in human gamete recognition.

Therefore, Whitmarsh downplays the importance of properly glycosylated rhZP3 protein and does not disclose or suggest a method of determining sperm activity using glycosylated rhZP3 expressed by a human ovarian cell.

To cure the deficiencies of Whitmarsh, the Examiner asserts that Chapman and Barratt disclose the importance of ZP3 glycosylation in sperm binding and mammalian fertilization, and therefore it would have been obvious to one of ordinary skill to utilize glycosylated rhZP3 as described by Chapman and Barratt in the method of Whitmarsh. Applicants respectfully submit that the Chapman and Barratt reference does not apply to the instant invention. Present claim 1 describes a method of determining human sperm activity using glycosylated recombinant *human* zona pellucida protein 3. The Examiner asserts that Chapman and Barratt teach the importance of ZP3 glysosylation lacking in Whitmarsh, and to support this assertion, cites to studies summarized on page 768, 1st column, 2nd paragraph ("Glycosylation and Fertilization") showing that the sperm-binding capacity of *mouse* ZP3 resides within the O-glycosylation present on the ZP3 protein.

Applicants submit that studies of mouse ZP3 glycosylation do not, as the Examiner claims, support the inference that ZP3 glycosylation is important to the sperm-binding capacity of *human* ZP3. Chapman and Barratt themselves state that although the mouse studies may be conclusive, studies in other mammals, especially humans, are not – following the description of the mouse studies cited by the Examiner, Chapman and Barratt state: "The data concerning the involvement of either *N*- or *O*- linked glycosylation in other mammals is equivocal" (page 769, 1st column, 2nd paragraph). Specifically with regard to the properties of human ZP3, Chapman and Barratt state: "Data relating to a role for carbohydrate in human sperm-zona binding are rare due to the lack of native zonae pellucidae" (page 769, 1st column, 3rd paragraph). Chapman and Barratt then describe the difficulties encountered in their own as well as other laboratories in attempting to purify biologically active human recombinant ZP3 (page 769, 1st column, 3rd

paragraph). They theorize that "characterization of active glycosylated recombinant human ZP3 is made difficult due to the fact that a number of different glycoforms have the potential of being synthesized depending on which cell line is chosen to express the ZP3 cDNA" (page 769, 1st column, 3rd paragraph). As to whether human gamete interaction utilizes a spermatozoa-based receptor protein to recognize and bind to a ZP3 carbohydrate epitope, as was shown in mice, Chapman and Barratt state that "at present one can only speculate" (page 769, 2nd column, 2nd paragraph).

Contrary to the Examiner's assertions, the Chapman and Barratt reference does not describe the importance of glycosylation in human ZP3. In fact, in order to ascertain the role of glycosylation in human ZP3, Chapman and Barratt attempted to produce recombinant non-glycosylated human ZP3, in an *E. coli* expression system. Chapman and Barratt used a bacterial expression system because it would produce a "more defined population of ZP3 molecules that are uniformly non-glycosylated" (page 772, column 1, paragraph 3). Chapman and Barratt suggested that "[i]f *E. coli*-derived ZP3 alone binds to human spermatozoa, it would suggest that glycosylation of ZP3 is not vital for successful human gamete interaction" (page 772, column 1, last sentence). The Examiner asserts that in their findings, Chapman and Barratt found that rhZP3 was immobilized on agarose beads, which bound sperm and induced the acrosome reaction (page 772, 2nd column, last 6 lines). However, this part of the reference cited by the Examiner again refers to *non-glycosylated* recombinant human ZP3 that Chapman and Barratt produced in their lab, using an in vitro transcription and translation system to avoid the folding problems of the recombinant *E. coli* proteins (page 772, 2nd column, last paragraph: "In vitro translated ZP3 is not glycosylated.").

One skilled in the art would not have a reasonable expectation of success that biologically active, glycosylated recombinant human ZP3 could be produced in the lab after reading Chapman and Barratt. Chapman and Barratt did not even attempt to produce a uniform population of fully glycosylated rhZP3, in a human ovarian cell system, bacterial cell system, or any other host system. By attempting to produce a uniform population of non-glycosylated rhZP3, Chapman and Barratt were trying to show that glycosylation is not important to ZP3 biological activity. At most, Chapman and Barratt describe the importance of ZP3 glycosylation in sperm binding and mammalian fertilization in mouse models. In contrast, the present inventors were able, for the first time, to produce biologically active, fully glycosylated recombinant human ZP3, from a human ovarian cell line.

The Examiner asserts that Whitmarsh in view of Chapman and Barratt differs from the instant invention in not specifically using rhZP3 expressed from human ovarian cells. However, as discussed above, the rhZP3 of Whitmarsh in view of Chapman and Barratt differs from the rhZP3 of the instant invention not only in that the recombinant ZP3 of the instant invention is expressed from human ovarian cells, but also in that the recombinant ZP3 of the instant invention is glycosylated human ZP3. The addition of the Franken reference does nothing to cure any of these deficiencies. The Examiner cites Franken for describing the expression of human ZP from oocytes derived from postmortem ovarian material, referring to the abstract and page 1010 of the Franken reference. Applicants respectfully submit that this disclosure does not, in fact, disclose or suggest rhZP3 recombinantly *expressed* from a human ovarian cell. Franken merely isolated ZP3 protein from postmortem ovarian material and then used this isolated protein in sperm-binding studies. However, Franken did not culture live human ovarian cells and use these cells

to *express* a ZP3 protein. Therefore, based on Franken, it would not have been obvious to one of ordinary skill in the art to express rhZP3 protein from a human ovarian cell.

Furthermore, one of ordinary skill in the art would not have had a reasonable expectation of success in expressing a biologically active rhZP3 from a human ovarian cell. As described in the present application at page 27, because hZP3 has a strong hydrophobic backbone, as well as large carbohydrate side chains, it is extremely difficult to produce the hZP3 glycoprotein by recombinant DNA technology. As described in the specification on page 27, various groups (including Whitmarsh et al.), have attempted to produce rhZP3; however, no one has been able to produce rhZP3 with full biological activity as measured by the ability to bind sperm and to induce an acrosome reaction. Applicants, for the first time, have produced rhZP3 which possesses both the ability to bind human sperm, as well as the ability to induce an acrosome reaction, in a manner similar to that of native hZP3.

Hence, Whitmarsh in view of Chapman and Barratt and further in view of Franken does not disclose the expression of glycosylated recombinant human ZP3 from human ovarian cells. Therefore, the combination of Whitmarsh, Chapman and Barratt, and Franken does not disclose the binding assay of claims 1 or 9. Accordingly, the present invention would not have been obvious to one of ordinary skill in the art and Applicants respectfully request that the rejection of claims 1 and 9 be withdrawn.

II. Rejection of claims 2-8 under 35 U.S.C. § 103(a)

Claims 2-8 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Whitmarsh et al. (Molecular Human Reproduction, Vol. 2, No. 12, pgs. 911-919) in view of

Chapman and Barratt (Molecular Human Reproduction, Vol. 2, No. 10, pgs. 767-777, 1996) and further in view of Franken et al. (Fertility and Sterility, Vol. 66, No. 6, December 1996). The Examiner asserts that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the concentration of the reagents to the specific concentrations recited in claims 2-8. Applicants respectfully traverse this rejection.

Whitmarsh in view of Chapman and Barratt and further in view of Franken does not disclose or suggest the binding assay of claim 1 for the reasons discussed above. Therefore, since the cited combination of references does not disclose or suggest the binding assay of the presently claimed invention, the various concentrations of the human ZP3 protein in claims 2-8 would not have been obvious to one of ordinary skill in the art at the time the invention was made. Applicants respectfully request that the rejection be withdrawn.

III. Rejection of claim 19 under 35 U.S.C. § 103(a)

The Examiner has rejected claim 19 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Whitmarsh et al. in view of Chapman and Barratt and further in view of Franken et al. and Foster et al. (U.S. Patent No. 4,444,879). Foster et al. is relied on in the Action for teaching kits including reactant reagents, a micro plate, positive controls, negative controls, standards, and instructions. The Examiner asserts that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to take the binding/detection assay as taught by Whitmarsh in view of Chapman and Barratt and Franken and format them into a kit because Foster et al. teach that it is convenient to do so and one can enhance sensitivity of a method by providing reagents as a kit.

Applicants respectfully submit that Whitmarsh does not disclose or suggest the claimed invention for the reasons set forth above. In particular, Whitmarsh does not disclose or suggest a diagnosis kit comprising glycosylated rhZP3 expressed from a human ovarian cell. Whitmarsh teaches away from the importance of glycosylation in sperm binding and provides no suggestion or disclosure of the importance of the expression of rhZP3 from a human ovarian cell in order to possess full biological activity.

Chapman and Barratt do not cure the deficiencies of Whitman. Chapman and Barratt also do not disclose or suggest the diagnosis kit of the presently claimed invention. Chapman and Barratt do not demonstrate the expression, or even the feasibility of expression, of glycosylated recombinant human ZP3 from a human ovarian cell. Therefore, Chapman and Barratt do not provide any disclosure or suggestion of a diagnosis kit comprising a glycosylated rhZP3 expressed from a human ovarian cell.

Franken also fails to cure the deficiencies of Whitman. Franken does not disclose or suggest the diagnosis kit of the present invention.

The Foster patent does not remedy the deficiencies of Whitmarsh, Chapman and Barratt, or Franken. The Foster patent discloses an assay reagent kit comprising a microtiter plate, a supply of various immunoglobins such as IgE, buffer wash solutions, enzyme-labeled anti-Ig conjugate, enzyme specific substrate, positive and negative controls, standards and instructions. The Foster patent does not describe or suggest a diagnostic kit for sperm activity comprising compartments with glycosylated recombinant human ZP3, expressed from a human ovarian cell, and one or more reagents listed in claim 19.

Accordingly, Applicants assert that none of the references, taken alone or in combination, describe or suggest the presently claimed invention of claim 19. Applicants respectfully request that the rejection be withdrawn.

IV. CONCLUSION

In view of the foregoing remarks, Applicants believe that the application is in condition for allowance. However, if the Examiner disagrees, she is encouraged to call the undersigned at the number listed below in order to expedite the prosecution of this application.

Respectfully submitted,

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